REMARKS

Claims 18, 19, 21-35, 37-49, 62, 63, and 65-73 are pending. Support for the amendment language is discussed in the appropriate sections below. Upon entry of the amendments set out herein, claims 18, 19, 21-35, 37-49, 62, 63, and 65-73 will be pending.

Applicant submits herewith a copy of WO 04/048528, as per the note in the Office Action that this reference was not considered because a copy was not provided. Applicants respectfully request consideration of this reference.

Independent claims 18, 34 and 62 are amended herein to recite "wherein the same set of distinguishably labeled primers determines the identities of the nucleotides at all members of the set of target polymorphic sites." Support for the amendment language is found, for example, on page 44, lines 5-11, which recite:

> The method detailed in this example can be further multiplexed by including an additional upstream primer extension primer for each additional SNP, having the same upstream tag and a 3' region specific for a different SNP-containing fragment of a distinct size from those already included. Each additional SNP interrogated must also have its own set of 4 downstream primers *carrying the same set of 4 downstream primer tags*, a 3' region that specifically hybridizes adjacent to the SNP, and a variable 3'-terminal nucleotide that corresponds to the tag sequence.

Claims 38 and 66 are amended herein to replace "comprising" with "comprises" to correct a grammatical error.

Rejections under 35 USC § 112, 2nd paragraph

Claims 43, 62, 63, and 65-73 stand rejected under 35 U.S.C. 112, second paragraph as being indefinite. The Office Action states on page 4, first paragraph:

> "Claim 43 is indefinite, because it recites the limitation "said tag sequence" in line 1. There is insufficient antecedent basis for this limitation in the claim"

Claim 43 is amended herein to recite "said first and/or said second tag sequences" to address the improper antecedent basis issue. The amendment language is supported throughout

the specification, but specifically on page 17-18, paragraph bridging pages 17-18, lines 1-9; and page 34, paragraph 2, lines 1-2.

The Office Action states on page 4, second paragraph:

> "Claims 62, 63, and 65-75 are indefinite, because independent claim 62 recites the limitation "said set of second oligonucleotide primers" in step VII. There is insufficient antecedent basis for this limitation in the claim."

Claim 62, Step VII is amended to recite "said set of downstream primers" instead of "said set of second oligonucleotide primers." Support for the amendment is found in the context of the original claim.

The Office Action states on page 4, third paragraph:

> "Claim 73 is further indefinite, because it recites the limitation "said set of distinguishably labeled downstream amplification primers" in lines 1-2. There is insufficient antecedent basis for this limitation in the claim."

Claim 62 is also amended herein to refer to "a set of *distinguishably labeled* downstream amplification primers, each member of said set of distinguishably labeled downstream amplification primers comprising..." Support for the amendment comes from the context of the original claim. This amendment is also sufficient to overcome the rejection under 35 U.S.C. 112 with regard to Claim 73.

Applicant submits that these amendments are sufficient to overcome the rejections under 35 U.S.C. 112, second paragraph. Reconsideration and withdrawal of the rejections is respectfully requested.

<u>Examiner's Response to Amendment regarding Myakishev et al.</u>

The previous Office Action response included a Declaration by Dr. Vladimir Slepnev addressing the Myakishev et al. reference alone and in combination with other cited references. In regard to the declaration filed by Dr. Slepnev, the present Office Action states on page 20, paragraph 2:

> "However, the declaration under 37 CFR 1.132 filed on January 9, 2008 is insufficient to overcome the rejections of claims 18, 19, 21-35, 37-49, 62, 63, and 65-73 made under 35 U.S.C. 103(a) citing Myakishev as the primary reference, because: (i) the showing is not commensurate in scope with the claims, and (ii) the facts presented are not germane to the rejection at issue. In points 6 and 7 of the declaration Dr. Slepnev argues that the claimed method is directed to the simultaneous amplification and detection of multiple different polymorphic sites (specifically SNPs) in a sample, and that one of ordinary skill in the art would not have a reasonable expectation of success in combining the teachings of Myakishev, Piggee, and Nolan to obtain such a method. However, the claims do not require that the amplification is performed in a multiplexed fashion. As a result, Applicant's arguments regarding the ability of the method taught by Myakishev to be multiplexed are not germane to the rejection at issue, since the claimed method *does not require this step*. Also since the claims do not require multiplexed amplification, the showing made in points 6 and 7 of the declaration is not commensurate in scope with the claimed invention." (Emphasis added)

Applicant respectfully disagrees.

The previously amended claims require that the amplification is performed in a multiplexed fashion, which is indicated by the language of the previously amended claims that recite, for example, "the *identities of the nucleotides* at a set of known polymorphic sites". The term "set" is defined in the specification on page 18, paragraph 4, line 2, which states "A set will comprise a known number of, and *at least two* of such entities." The plain language of the claims also makes it clear that a plurality of polymorphic sites is assayed in one reaction where, e.g., claim 18 recites "detecting incorporation of a distinguishable label in distinctly sized amplification products, thereby to determine the identity of the nucleotide at *each* said polymorphic site." Thus, amendment to specify multiplex is not required.

However, to further emphasize the multiplex aspect of the claims, step (a) of Claim 18 is herein amended to recite "subjecting to a multiplex amplification regimen a population of primer extension products generated from a nucleic acid sample, each primer extension product comprising a member of a set of tag sequences, which tag sequence specifically corresponds to the presence of one specific nucleotide *of a member of said set of known polymorphic sites...*" All other independent claims are similarly amended herein to further highlight that the claims are directed to the multiplex identification of a plurality of polymorphic sites. Support for the

amendments is found throughout the specification and particularly at, for example, page 5, paragraph 1, lines 7-10; page 24, paragraph 5, lines 1-3; page 25, paragraph bridging pages 25-26, lines 9-10; page 30, paragraph 3, lines 1-3; page 31, first paragraph, lines 16-18; and page 31, paragraph 2, line 1. In view of the clear multiplex requirements in the claims, and in view of Dr. Slepnev's earlier declaration, Applicant submits that the claimed invention is patentably distinguished over the teachings of Myakishev et al., alone or in combination with the further references cited.

Applicant respectfully requests reconsideration of the claims as amended, in view of Dr. Slepnev's declaration. Further discussion of the particular rejections is provided below.

Rejections under 35 U.S.C. § 103

Myakishev et al. in view of Piggee et al.

Claims, 18, 19, 21, 22, 26, 27, 34, 35, 37, 38, 42, and 43 stand rejected under 35 U.S.C. 103(a) based on the combination of Myakishev et al., in view of Piggee et al..

The Office Action states "Regarding claim 18, Myakishev teaches a method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated (see Figure 2 and pages 163-165 where nine SNPs were tested." While Myakishev et al. may describe application of their method to nine different SNPs, at no time was more than one SNP interrogated in the same reaction. That is, Myakishev et al. does not teach multiplex detection. Not only does Myakishev et al. not teach multiplex, but as discussed in Dr. Slepnev's Declaration, the Myakishev et al. method is not amenable to multiplexing, at least in part because the different fluorescence spectra of labels needed for each target preclude it.

Further, Myakishev et al. teaches that it is an advantage of the method they describe that "the reactions are run in a single tube in a generic PCR machine and read in a standard fluorescence plate reader." (p. 163) At page 167 the reference states "A final advantage of the method is the small number of operations that are involved. DNA and reagents are mixed in multiwell PCR plates, the amplification reactions are run in multiple standard thermocyclers, the plates are transferred and read in a fluorescence plate reader, and the data are converted into

genotypes by a computer program." Myakishev et al. thus teaches away from an approach that requires further steps. The homogeneous aspect of the assay is an important part of the operating principle – reading on a plate reader, rather than adding another operation is important, and the reference addresses the potential for high throughput by stressing the use of multiwell plates, with multiple *separate* reactions performed in parallel, rather than in multiplex. Not only does Myakishev et al. stress the ability to perform multiple separate reactions in multiwell plates to increase throughput, thereby negating a need to multiplex, the proposed combination with the teachings of capillary electrophoresis in Piggee et al. for multiplex would necessarily add a new operation, the capillary electrophoresis step, requiring further equipment and sample manipulation. The approach for increasing throughput proposed in the Office Action thus goes counter to an express advantage taught by Myakishev et al. and would not therefore be a proper combination under the law.

Piggee et al. is cited as teaching the use of primers of different length for multiplex detection. Specifically, the reference states "By using multiplex detection, several different point mutations could be detected in the same reaction tube by different length primers." The proposed use of primers of different lengths does not mean that the amplified sequences as taught by Myakishev et al. will be different lengths. As discussed previously, Piggee et al. does not teach amplification at all, let alone amplification resulting in distinctly sized products. Also, Piggee et al. does not use distinguishably labeled primers – even the mentioned primers of different lengths are not provided in labeled form in the method of Piggee et al.

Further, and importantly, the claimed methods can use the *same set of tag-specific primers to detect all different SNPs in a multiplex reaction* – by definition then, the distinguishably labeled primers used in the claimed method will *not* be of different lengths for different targets. (Independent claims 18, 34 and 62 are amended herein to further emphasize this distinction, each reciting "wherein the same set of distinguishably labeled primers determines the identities of the nucleotides at all members of the set of target polymorphic sites," as noted above.) For example, the primer in the claimed methods that specifically amplifies the primer extension product tagged with an A-specific tag will detect each member of the set of target SNPs that has an A at the polymorphic site. That is, a single primer of a single length will detect the A allele for any number of different target SNPs. The proposed combination of the different length primers of Piggee et al. with the teachings of Myakishev et al. does not therefore

result in the invention as claimed, e.g., in claims 18, 34 or 62 or claims that depend from them. In order to use primers of different lengths to multiplex the method of Myakishev et al., different length primers for each different SNP target in a set would be required. This is clearly not what is recited in the claimed methods, and the proposed combination of Myakishev et al. with Piggee et al. therefore fails to teach all elements of the claimed invention.

Applicants submit that the invention as currently claimed could not be derived by any combination of Myakishev et al. and Piggee et al., and respectfully request reconsideration and withdrawal of this rejection under 35 U.S.C. § 103.

Myakishev et al. in view of Piggee et al. and further in view of Woolley et al.

Claims 23-25 and 39-41 stand rejected under 35 U.S.C. 103 based on the combination of Myakishev et al., and Piggee et al., and further in view of Woolley et al. Myakishev et al. and Piggee et al. are cited as above, and Woolley et al. is cited for an integrated PCR-CE microdevice for use in the method resulting from the combined teachings of Myakishev et al. and Piggee et al.

Claims 23-25 ultimately depend from independent claim 18, and claims 39-41 ultimately depend from independent claim 34. As discussed above, the combination of Myakishev et al. and Piggee et al. does not teach all elements of either of claims 18 or 34 as amended. The addition of the teachings of Woolley et al., whether it teaches an integrated PCR-CE microdevice or not, does not remedy the defects of the primary references in this regard. If the combination does not render the independent claims obvious, it cannot render the dependent claims obvious either.

Applicant respectfully requests that the rejections under 35 U.S. C. § 103 based on Myakishev et al., Piggee et al. and Woolley et al. be reconsidered and withdrawn.

Myakishev et al. in view of Piggee et al., and further in view of Nolan et al.

Claims 28-33, 44-49, 62, 63, 65, 66, and 69-73 stand rejected under 35 U.S.C. 103(a) based on the combination of Myakishev et al., and Piggee et al., further in view of Nolan et al.. The Office Action states that Nolan et al. teaches that "unincorporated primers from an initial

amplification reaction are degraded using the heat labile Exonuclease I followed by polymerase extension." (Page 16, paragraph 4, lines 2-4)

As discussed above, the combination of Myakishev et al., and Piggee et al., does not teach all of the elements of the invention as presently claimed in independent claims 18, 34 or 62, which each require multiplex amplification for the genotyping of a set of SNPs in one reaction. The combination of Myakishev et al. and Piggee et al. also fails to teach a method in which "the same set of distinguishably labeled primers determines the identities of the nucleotides at all members of the set of target polymorphic sites," as required by each of these claims as amended. The rejection includes claim 62 and claims dependent from claims 18 and 34. The addition of the Nolan et al. reference does not correct these deficiencies of the proposed combination of Myakishev et al. with Piggee et al. That is, the teaching of the removal of unincorporated primers does not remedy the lack of a multiplex amplification reaction in either of Myakishev et al. or Piggee et al., the lack of amplification of a set of primer extension products, or the lack of a method in which the same set of distinguishably labeled primers determines the identities of the nucleotides at all members of the set of target polymorphic sites. Therefore, Applicants submit that the combination of Myakishev et al., Piggee et al., and Nolan et al., does not teach all of the elements of the invention as presently claimed. Applicants respectfully request that the rejection under 35 U.S.C. 103 regarding this combination of references be withdrawn.

In view of the above, all issues raised in the Office Action mailed November 12, 2008 have been addressed herein. Reconsideration of the claims is respectfully requested.

A Petition for Extension of Time and authorization to charge the necessary fees to Deposit Account No. 50-0850 are filed herewith. Authorization is hereby given to charge any further fees necessary or to credit any overpayment to this deposit account, referencing Docket No. 046264-065331.

Respectfully submitted:

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